

**SYNTHESIZE AND CHARACTERIZATION OF
BIOACTIVE GLASS POWDER BASED ON
SiO₂-CaO-Na₂O-P₂O₅ SYSTEM**

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UNIVERSITI SAINS MALAYSIA

2019

**SYNTHESIZE AND CHARACTERIZATION OF BIOACTIVE GLASS
POWDER BASED ON SiO_2 -CaO- Na_2O - P_2O_5 SYSTEM**

by

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**Thesis submitted in fulfilment of the
requirements for the degree of
Doctor of Philosophy**

March 2019

ACKNOWLEDGEMENTS

Alhamdulillah. Praise to Allah The Almighty. The research and thesis would never have been completed without His guidance. Thanks to my husband as willing to engage with the struggle since the day I decided to pursue my studies. Thank you for your practical and emotional support. I would like to express my sincere gratitude to my mentor and supervisor, Assoc. Prof. Dr. Hasmaliza Mohamad for her excellent cooperation, guide, continuous support, tolerance, motivation and all opportunities given during my research and writing of thesis. It was a great pleasure working with her for the past few years since undergraduate.

Special thanks to my co-supervisor, Assoc. Prof. Dr. Siti Noor Fazliah Mohd Noor for the support and the access given to the laboratory and research facilities in Advanced Medical & Dental Institute, USM. Sincere thanks also go to my co-supervisor Nurazreena Ahmad for the moral support. I also wish to express my sincere thanks to Dean of SMMRE, all lectures, administrative and technical staffs in School of Materials and Mineral Resources Engineering, USM for the helps and assistances during the research. Great thanks to the lab mates, particularly Nurul Shazwani Mohd Zain for the discussion, help and fun during the research.

Lastly, I would like to thank my parents, my sister and brother for the inspiration and moral support for the research completion.

May Allah bless all of you. Amen.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	viii
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xv
LIST OF SYMBOLS	xvi
ABSTRAK	xvii
ABSTRACT	xviii
CHAPTER ONE : INTRODUCTION	
1.1 Biomaterials	1
1.1.1 Bioactive glass	2
1.1.2 45S5 Bioglass	4
1.2 Problem statement	6
1.3 Research objective	8
1.4 Scope of research	9
CHAPTER TWO : LITERATURE REVIEW	
2.1 Ceramic as biomaterials	11
2.2 Biomaterial	12
2.2.1 Bioglass	14
2.3 Bioactive material	16

2.4	Glass as a biomaterial	18
2.4.1	Glass formation	20
2.5	Bioactive glass	23
2.5.1	Synthesize of bioactive glass	26
2.5.1(a)	Melt derived	27
2.5.1(b)	Sol gel	31
2.5.2	Bioactive Glass Composition	33
2.5.2(a)	Silicon dioxide (SiO_2)	37
2.5.2(b)	Sodium oxide (Na_2O)	39
2.5.2(c)	Calcium Oxide (CaO)	41
2.5.2(d)	Phosphorus Pentoxide (P_2O_5)	43
2.5.3	Network connectivity (Nc) of bioactive glass	44
2.5.4	Bioactive glass surface reactions	46
2.5.5	Growth of apatite layer on the surface of bioactive glass	47
2.5.6	Bioactivity assessment of bioactive glass	49
2.6	Bioactive glass application	52
2.7	Summary	55

CHAPTER THREE : MATERIALS AND METHODOLOGY

3.1	Introduction	56
3.2	Raw materials	56
3.3	Methodology	57
3.3.1	Synthesize of bioactive glass powder	57
3.3.1(a)	Batching	58
3.3.1(b)	Melting	62

3.3.1(c)	Quenching	63
3.3.1(d)	Drying	64
3.3.1(e)	Milling	64
3.3.1(f)	Sieving	64
3.4	Characterizations	66
3.4.1	X-ray fluorescence (XRF)	66
3.4.2	Particles size analysis (PSA)	67
3.4.3	Different Scanning Calorimetric (DSC)	67
3.4.4	X-ray diffraction analysis (XRD)	68
3.4.5	Fourier transform infrared analysis (FTIR)	69
3.4.6	Scanning electron microscopy (SEM) & Energy-Dispersive X-ray (EDX)	69
3.4.7	<i>In vitro</i> test	70
3.4.8	Elemental analysis of ionic BG using ICP-OES	71
3.4.9	pH measurement analysis	71
3.4.10	Cell culture	72
3.4.11	Variability Gauge Chart	72
3.4.12	Oneway analysis	74

CHAPTER : FOUR RESULTS AND DISCUSSION

4.1	Introduction	76
4.2	Characterization of raw materials	76
4.2.1	Silicon dioxide (SiO ₂)	76
4.2.2	Calcium carbonate (CaCO ₃)	77
4.2.3	Sodium carbonate (Na ₂ CO ₃)	78

4.2.4	Phosphorus pentoxide (P ₂ O ₅)	79
4.3	Synthesize of BG powder with different composition	80
4.3.1	Physical appearance	80
4.3.2	Thermal analysis	82
4.3.3	XRF analysis	86
4.3.4	Particle size analysis	88
4.3.5	XRD analysis	90
4.3.6	FTIR analysis	93
4.3.7	SEM and EDX analysis	95
4.4	Synthesize of BG powder at different melting temperature and soaking time	97
4.4.1	Physical appearance	98
4.4.2	XRF analysis	99
4.4.3	Particle size analysis	103
4.4.4	XRD analysis	109
4.4.5	FTIR analysis	112
4.5	Bioactivity evaluation <i>via in vitro</i> test using Tris buffer solution	115
4.5.1	XRD analysis	116
4.5.2	FTIR analysis	123
4.5.3	pH analysis	132
4.5.4	SEM and EDX analysis	137
4.5.5	Ion release analysis	149
4.6	Bioactivity evaluation <i>via in vitro</i> test using simulated body fluid (SBF)	153
4.6.1	XRD analysis	153
4.6.2	FTIR analysis	155

4.6.3	pH analysis	159
4.6.4	SEM and EDX analysis	161
4.6.5	Ion release analysis	165
4.7	Reaction of bioactive glass powder in cell culture	167
 CHAPTER : FIVE CONCLUSIONS		
5.1	Conclusion	170
5.2	Recommendation for Further Work	171
REFERENCES		173
APPENDICES		

LIST OF TABLES

	Page
Table 2.1 Various composition of BG available in the laboratory	34
Table 2.2 Example of melt derived bioactive glass and its network connectivity	46
Table 2.3 The different stages in bone development on BG surface	49
Table 3.1 Raw materials and their properties that have been used in synthesized of bioactive glass powder	57
Table 3.2 The bioactive glass composition used in this research	58
Table 3.3 Oxides composition for synthesized 45S5 bioactive glass powder	59
Table 3.4 Oxides composition for synthesized 50S8P bioactive glass powder	59
Table 3.5 Oxides composition for synthesized 54S4P bioactive glass powder	60
Table 3.6 Oxides composition for synthesized 46S0P bioactive glass powder	60
Table 3.7 Minimum and maximum in weight percentage (wt. %) of each oxides use to synthesize bioactive glass	62
Table 3.8 Melting temperature (°C) and soaking time for each bioactive glass composition	63
Table 4.1 Elemental analysis (wt. %) of SiO ₂ by XRF	77
Table 4.2 Elemental analysis (wt. %) of CaCO ₃ by XRF	78
Table 4.3 Elemental analysis (wt. %) Na ₂ CO ₃ of by XRF	79
Table 4.4 Mass reduction (wt. %) over temperature (°C)	85
Table 4.5 Average particle size (µm) for different composition of milled BG powder	89
Table 4.6 Average particle size for milled 45S5 synthesized at different temperature and soaking time	104

Table 4.7	Average particle size for milled 50S8P synthesized at different temperature and soaking time	105
Table 4.8	Average particle size for milled 54S4P synthesized at different temperature and soaking time	106
Table 4.9	Average particle size for milled 46S0P synthesized at different temperature and soaking time	107

LIST OF FIGURES

		Page
Figure 1.1	General experimental procedure to synthesize BG powder	10
Figure 2.1	Illustration on two dimensional structures of SiO ₂	19
Figure 2.2	Diagram on the effects of temperature towards glass formation with function of temperature and enthalpy	21
Figure 2.3	Surface reactions induced by different types of glasses	26
Figure 2.4	Liquidus surfaces and equilibrium phase in system SiO ₂ -CaO-Na ₂ O	29
Figure 2.5	The process of synthesize BG powder through melt derived route	30
Figure 2.6	The process flow to synthesize BG powder through sol gel route	32
Figure 2.7	Compositional dependence (in wt. %) for bone bonding and soft tissue bonding for bioactive glass and glass ceramic	35
Figure 2.8	Two dimensional structure of silicon tetrahedral	39
Figure 2.9	Two dimensional structure of silicate glass with sodium oxide as network modifier	41
Figure 2.10	Two dimensional structure of silicate glass with sodium and calcium oxide as network modifier	42
Figure 2.11	Schematic diagram on the interface reaction between glass and solution on calcium phosphate (Ca-P) formation	47
Figure 2.12	Surface morphologies of 45S5 BG via SEM	50
Figure 2.13	XRD pattern of glass after immersion in Tris solution for several days	51
Figure 2.14	FTIR spectra of 45S5 BG after immersion in SBF after one day	51
Figure 2.15	SEM images for BG particles produced by NovaBone	54
Figure 3.1	Correlation between moving factor and the output factor	61

Figure 3.2	Moving factor design based on minimum and maximum percentage of each oxide (wt. %) using software JMP PRO 12	62
Figure 3.3	Melting profile for synthesized BG powder	63
Figure 3.4	Process flow on synthesized BG powder	65
Figure 3.5	The procedure during in vitro evaluation	71
Figure 3.6	Examples of variability chart (comparison graph)	73
Figure 3.7	Variability chart window	74
Figure 3.8	Example of oneway analysis	74
Figure 3.9	Oneway analysis chart window	75
Figure 4.1	XRD pattern of SiO_2 powder	77
Figure 4.2	XRD pattern of CaCO_3 powder	78
Figure 4.3	XRD pattern of Na_2CO_3 powder	79
Figure 4.4	XRD pattern of P_2O_5 powder	80
Figure 4.5	Image of BG frit obtained after quenched	81
Figure 4.6	DSC result for different composition of BG powder	83
Figure 4.7	TGA result for different composition of BG powder	86
Figure 4.8	Comparison between XRF and calculated weight on oxides for all BG composition	87
Figure 4.9	Particle size distribution for milled BG powder	88
Figure 4.10	XRD pattern for all BG composition after melting	91
Figure 4.11	Scatter plot matrix for XRD with different BG composition	92
Figure 4.12	FTIR spectrum for all BG composition after melting	94
Figure 4.13	SEM (5kX mag) and EDX result for BG powder after milling	96
Figure 4.14	Image of BG frit	98
Figure 4.15	Comparison between XRF and calculated weight on 45S5 BG powder	100

Figure 4.16	Comparison between XRF and calculated weight on 50S8P BG powder	101
Figure 4.17	Comparison between XRF and calculated weight on 54S4P BG powder	101
Figure 4.18	Comparison between XRF and calculated weight on 46S0P BG powder	102
Figure 4.19	Particle size distribution for milled 45S5 BG powder	104
Figure 4.20	Particle size distribution for milled 50S8P BG powder	105
Figure 4.21	Particle size distribution for milled 54S4P BG powder	106
Figure 4.22	Particle size distribution for milled 46S0P BG powder	107
Figure 4.23	XRD pattern for 45S5 BG powder	110
Figure 4.24	XRD pattern for 50S8P BG powder	110
Figure 4.25	XRD pattern for 54S4P BG powder	111
Figure 4.26	XRD pattern for 46S0P BG powder	111
Figure 4.27	FTIR spectrum for 45S5P BG powder	113
Figure 4.28	FTIR spectrum for 50S8P BG powder	113
Figure 4.29	FTIR spectrum for 54S4P BG powder	114
Figure 4.30	FTIR spectrum for 46S0P BG powder	114
Figure 4.31	XRD pattern for 45S5 BG powder after immersion in Tris solution	116
Figure 4.32	XRD pattern for 50S8P BG powder after immersion in Tris solution	117
Figure 4.33	XRD pattern for 54S4P BG powder after immersion in Tris solution	118
Figure 4.34	XRD pattern for 46S0P BG powder after immersion in Tris solution	119
Figure 4.35	FTIR spectrum for 45S5 BG powder after immersion in Tris solution	125
Figure 4.36	FTIR spectrum for 50S8P BG powder after immersion in Tris	126

Figure 4.37	FTIR spectrum for 54S4P BG powder after immersion in Tris solution	127
Figure 4.38	FTIR spectrum for 46S0P BG powder after immersion in Tris solution	128
Figure 4.39	pH trend of Tris solution	132
Figure 4.40	SEM (10kX mag) and EDX result of BG powder after immersion for 7 days	137
Figure 4.41	SEM (10kX mag) and EDX result of BG powder after immersion for 14 days	140
Figure 4.42	SEM (10kX mag) and EDX result of BG powder after immersion for 21 days	143
Figure 4.43	SEM (10kX mag) and EDX result of BG powder synthesized at lower temperature after immersion for 7 days	145
Figure 4.44	SEM (10kX mag) and EDX result of BG powder synthesized at lower melting temperature after immersion for 14 days	146
Figure 4.45	SEM (10kX mag) images and EDX result of BG powder synthesized at lower melting temperature after immersion for 21 days	147
Figure 4.46	Ion dissolution trend from BG in Tris solution	150
Figure 4.47	XRD pattern of BG powder after immersion in SBF solution	154
Figure 4.48	FTIR spectrum of BG powder after immersion in SBF solution	156
Figure 4.49	pH trend of SBF solution	159
Figure 4.50	SEM (10kX mag) and EDX results of BG powder after immersion for 7 days	161
Figure 4.51	SEM (10kX mag) and EDX result of BG powder after immersion for 14 days	163
Figure 4.52	SEM (10kX mag) and EDX result of BG powder after incubation for 21 days	164
Figure 4.53	Ion dissolution trend from BG in SBF solution	166

Figure 4.54	Optical images (10X mag) of DPSC cell incubated with 45S5 BG powder	168
Figure 4.55	Optical images (10X mag) of DPSC cell incubated with 50S8P BG powder	168
Figure 4.56	Optical images (10X mag) of DPSC cell incubated with 54S4P BG powder	169
Figure 4.57	Optical images (10X mag) of DPSC cell incubated with 46S0P BG powder	169

LIST OF ABBREVIATIONS

BG	Bioactive glass
CTE	Coefficient of thermal expansion
DMEM	Dulbecco's Modified Eagle Medium
DPSC	Dental pulp stem cell
DSC	Differential scanning calorimetry
EDX	Energy dispersive x-ray
FTIR	Fourier transform infrared spectroscopy
FESEM	Field emission scanning electron microscopy
HA	Hydroxylapatite
HCA	Hydroxylcarbonate apatite
ICP-OES	Inductively coupled plasma optical emission spectroscopy
PDF	Powder diffraction file
PSA	Particle size analysis
RMM	Relative molecular mass
rpm	Revolutions per minute
SBF	Simulated body fluid
TE	Tissue engineering
TTT	Time temperature transformation
XRD	X-ray diffraction
XRF	X-ray fluorescence
45S5	45SiO ₂ -24.5CaO-24.5Na ₂ O-6P ₂ O ₅ (wt. %)
50S8P	50SiO ₂ -22CaO-20Na ₂ O-8P ₂ O ₅ (wt. %)
54S4P	54SiO ₂ -22CaO-20Na ₂ O-4P ₂ O ₅ (wt. %)
46S0P	46SiO ₂ -24CaO-30Na ₂ O-0P ₂ O ₅ (wt. %)

LIST OF SYMBOLS

mol. %	Mole percentage
N _c	Network connectivity
T _c	Crystallization temperature
T _m	Melting temperature
T _g	Glass transition temperature
t	Time
%	Percentage
wt. %	Weight percentage
°	Degree
a.u	Arbitrary unit
θ	Incidence angle of X-ray beam

SINTESIS DAN PENCIRIAN SERBUK KACA BIOAKTIF BERASASKAN SISTEM $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$

ABSTRAK

Serbuk kaca bioaktif (BG) telah digunakan sebagai pengisi dalam kecacatan tulang kerana keupayaan untuk berhubung dengan tisu tulang melalui pembentukan ikatan dengan lapisan apatit. Walau bagaimanapun, suhu lebur yang lebih tinggi ($1450\text{ }^{\circ}\text{C}$ - $1570\text{ }^{\circ}\text{C}$) atau masa lebur yang lebih lama (3 jam) diperlukan untuk menghasilkan serbuk BG melalui kaedah lebur kaca konvensional. Dalam penyelidikan ini, komposisi baru (50S8P, $N_c = 2.69$), (54S4P, $N_c = 2.60$) dan (46S0P, $N_c = 1.62$) serbuk kaca bioaktif telah dibangunkan daripada sistem $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$ untuk mendapatkan sifat-sifat pemrosesan dan biologi yang baik. Penghasilan BG termasuk pengelompokan, pencampuran, peleburan pada suhu yang berbeza, lindap kejut air, pengisaran dan pengayakkan. BG dengan komposisi 45S5 digunakan sebagai kawalan. Pembelaun sinar-X (XRD) memperlihatkan struktur kaca amorfus sepenuhnya diperolehi untuk semua komposisi BG dengan puncak lebar antara $30\text{-}35^{\circ}$. Kaca berasaskan rangkaian silika juga disahkan melalui transformasi Fourier spektroskopi inframerah (FTIR) dengan kumpulan berfungsi Si-O-Si (tetrahedral) dikenalpasti dalam spektrum. Analisis haba membuktikan bahawa semua komposisi BG boleh dileburkan pada suhu lebih rendah iaitu 45S5 pada $1377\text{ }^{\circ}\text{C}$, 50S8P dan 54S4P pada $1348\text{ }^{\circ}\text{C}$ dengan 46S0P pada $1347\text{ }^{\circ}\text{C}$. Oleh itu, kesan suhu dan masa lebur yang berlainan (1.5, 1 dan 0.5 jam) juga dikaji. Berdasarkan XRD, struktur amorfus masih kekal walaupun serbuk BG dihasilkan pada suhu lebur yang lebih rendah pada 0.5 jam untuk semua komposisi BG. Bioaktiviti BG dinilai dengan pengeraman serbuk BG dengan larutan penimbal Tris (pH 8) selama 7, 14 dan 21 hari. Ujian *in vitro* mengesahkan pembentukan hidrosilapatit (HA) pada permukaan BG dengan kemunculan puncak berhablur dalam XRD dan ciri-ciri kumpulan berfungsi karbonat (C-O) dan fosfat (P-O) yang kelihatan dalam FTIR dengan keamatan puncak yang lebih tinggi telah diperhatikan pada 45S5 dan 50S8P BG berbanding dengan 54S4P dan 46S0P BG. Tindak balas biologi yang lebih baik diperhatikan pada BG yang dibuat pada suhu $1400\text{ }^{\circ}\text{C}$ setelah pengeraman dan seterusnya diuji dengan *in vitro* simulasi cecair badan (SBF), pH 7.3 dan media sel. Namun, ciri-ciri pengamatan HA yang kurang telah diperhatikan pada XRD dan FTIR pada permukaan BG setelah direndam dalam SBF berbanding dengan larutan penimbal Tris. Kebolehserasian yang baik diperhatikan apabila sel stem pulpa gigi (DPSC) didedahkan kepada semua komposisi BG. Kesimpulannya, komposisi baru serbuk BG telah berjaya dibangunkan pada suhu dan masa lebur yang rendah dengan sifat biologi yang baik walaupun mempunyai sambungan rangkaian yang tinggi.

SYNTHESIZE AND CHARACTERIZATION OF BIOACTIVE GLASS

POWDER BASED ON $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$ SYSTEM

ABSTRACT

Bioactive glass (BG) powder have been used as a filler in bone defects due to the ability to connect with bone tissue through bonding formation with apatite layer. However, higher melting temperature ($1450\text{ }^{\circ}\text{C}$ - $1570\text{ }^{\circ}\text{C}$) or longer soaking time (3 hours) is required to produce BG powder *via* conventional glass melting route. In this research work, new composition (50S8P, $N_c = 2.69$), (54S4P, $N_c = 2.60$) and (46S0P, $N_c = 1.62$) of bioactive glass powder was developed from $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$ system to obtain good processing and biological properties. The BG preparations included batching, mixing, melting at different temperature, water quench, milling and sieving. BG with 45S5 composition was used as a control. X-ray diffraction (XRD) revealed that fully amorphous glass structure was obtained for all BG composition with broad peaks between $30\text{-}35^{\circ}$. Silica network based glass was also confirmed through Fourier transform infrared spectroscopy (FTIR) with Si-O-Si (tetrahedral) functional group was observed in the spectrum. Thermal analysis proved that all BG composition can be melted at lower temperature where 45S5 at $1377\text{ }^{\circ}\text{C}$, 50S8P and 54S4P at $1348\text{ }^{\circ}\text{C}$ with 46S0P at $1347\text{ }^{\circ}\text{C}$. Hence, the effect of different melting temperature and time (1.5, 1 and 0.5 hour) were also studied. Amorphous structure was still retained based on XRD although BG powder was synthesized with lower melting temperature at shorter melting time, 0.5 hour for all BG composition. The BG bioactivity was evaluated by incubating the BG powder with Tris buffer solution (pH 8) for 7, 14 and 21 days. *In vitro* test confirmed on the hydroxylapatite (HA) formation on the BG surface with emerging of crystalline peaks in XRD. Characteristic of carbonate (C-O) and phosphate (P-O) functional group noticed in FTIR with more intense peaks was observed on 45S5 and 50S8P BG compared to 54S4P and 46S0P BG. Better biological responds was observed on BG synthesized at $1400\text{ }^{\circ}\text{C}$ upon incubation and was further evaluated by *in vitro* test in simulated body fluid (SBF), pH 7.3 and cell culture. However, less intense HA characteristic was observed in XRD and FTIR on the BG surface upon immersion in SBF compared to Tris buffer solution. Good compatibility was observed when dental pulp stem cell (DPSC) was exposed to all BG composition. In conclusion, new composition of BG powder was successfully developed at lower melting temperature and soaking time with good biological properties although possess high network connectivity.

CHAPTER ONE

INTRODUCTION

1.1 Biomaterials

A material that is able to interact with biological system by providing treatment, tissue or organ replacement and function of body is known as biomaterial. Initially, the first generation of biomaterial was focused on the mechanical performances of implant material and the material selection was limited to those that exhibit inert characteristics (Crovace *et al.*, 2016). Inert material such as steels, carbon materials, silicones, and poly (methyl methacrylate) were examples of biomaterials. These materials exhibit biocompatibility characteristic yet suffer on non-degradation properties which limit their usage in clinical applications (Wang, 2016). These provided the basis for the invention of second and third generation of biomaterials (Crovace *et al.*, 2016).

Development of biomaterials for clinical applications require some additional excellent important characteristic such as the ability of the biomaterial to be harmonized with micro-environment of defective tissue, ability to support the mechanical stability of defective tissue during tissue repair and possess the adaptable biodegradability characteristic which matches the new tissue formation (Wang, 2016). The second generation of biomaterials demonstrate such characteristic as the ability to induce reaction in the physiological environment. Meanwhile, the growth of third generation of biomaterials received great attention due to the capability of the biomaterials to stimulate specific cellular responses at the molecular stage and able to activate genes responsible for living tissue regeneration. Bioactive glass and glass ceramic are examples of third generation of biomaterials (Crovace *et al.*, 2016).

Biomaterials is widely used in clinical applications such as in repairing bone defects. Several reasons that induced bone defects are infection, trauma, tumor and congenital deformity. The autogenous bone transplantation is optimum alternative for the treatment. However this procedure has drawbacks such as limited source, might induce damage at the site of transplantation. The other option is to introduce biomaterials that have potential restorative effects in bone defects. Biomaterial such as bioactive glass (BG) is widely used to repair bone defect due to the ability to bond and integrate with bone in living body through rapid formation of apatite layer on the material surface upon exposure to biological environment (Mosbahi *et al.*, 2016). Biomaterials also being implemented in dental treatment applications. The use of bioactive glass in dental treatment enables the induction of remineralization and assist against local irritation. In addition, the possibility to use BG in periodontal disease treatment is also recognized. It was reported that the combination of BG and clodronate enhanced ion exchange resulting in apatite formation in dental application to treat periodontitis during maintenance phase (Rosenqvist *et al.*, 2014). Tooth sensitivity can also be treated using biomaterial such bioactive glass where the BG is added in tooth paste for the treatment (Fernando *et al.*, 2017). The use of biomaterial in wound healing treatment (Lv *et al.*, 2017) and bone fracture (Arcos *et al.*, 2014) due to osteoporosis also has been widely explored and studied.

1.1.1 Bioactive glass

A glass is a material which is obtained by heating a solid mixture material until it reaches a viscous state and quickly cooled to prevent the formation of crystalline structure. Upon quenching, the atom remains in the disordered state characteristic of liquids (Salinas, 2014). The glass structure exhibit random array of atoms and are linked by directional bonding and glass network which contains no

regular pattern to the spacing among neighbour, and thus are often called ‘amorphous’ or without form (Kelly and Benetti, 2011; Wright, 2014). The open structure of amorphous glass facilitates the inclusion of network modifiers inducing the discontinuity of the glass network. The disordered structure leads to the high reactivity of glass in aqueous environments. The high surface reactivity of glass is the prime advantages of their application in bone repair and replacement (El-Kheshen *et al.*, 2008).

The use of bioactive glass (BG) has received a great attention for bone and dental treatment since its first invention by Hench in the 1970s. The primary characteristic of BG which includes the ability to integrate with living tissue has induced rapid development of BG in biomedical applications (Rahaman *et al.*, 2011). Formerly, implant material exhibit only bioinert character and tends to evoke undesirable fibrous encapsulation around the material upon implantation. However, BG demonstrates a positive response upon implantation by developing a stable bond and interface with living tissue without formation of contact between fibrous tissue and the living tissue. The bond between BG and tissue is formed through apatite layer formation (Miguez-Pacheco *et al.*, 2015).

The important characteristics of BG such as high bioactivity which has the ability to develop hydroxyapatite layer on the glass surface, osteoconduction and osteostimulation make them suitable to be used for bone and tooth repair regeneration (Miguez-Pacheco *et al.*, 2015). The bioactivity depends on the ability to develop a bone-like mineral on the glass surface when in contact with physiological fluids (Orgaz *et al.*, 2016). BG also shows excellent osteogenic characteristic due to ion released during glass dissolution which has the ability to stimulate expression of numerous genes that promote osteoblastic cell proliferation

and differentiation (Xynos *et al.*, 2001; Hench, 2009). The classic application of BG includes bone filling materials, bioactive coating on orthopedic implant, dental applications and small bone implant (Jones, 2015).

1.1.2 45S5 Bioglass

The first bioactive glass was synthesized based on $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$ system. The classical bioactive glass is generally referred as 45S5 Bioglass® and originally developed by Hench. The glass is characterized in nominal composition of 45% silicon dioxide (SiO_2), 24.5% calcium oxide (CaO), 24.5% sodium oxide (Na_2O) and 6% phosphorus pentoxide (P_2O_5) (in weight percent) (Desogus *et al.*, 2015). The unique glass composition was reported as 45S5 to indicate the weight percentage of silica (SiO_2) used as the network former and a 5-fold ratio of Ca/P (Hench, 2013). The lower content of network former silicon dioxide (SiO_2) and higher content of glass network modifier, sodium oxide (Na_2O) and calcium oxide (CaO) is the key feature that contributes in the bioactivity of 45S5 glass (Rahaman *et al.*, 2011). P_2O_5 was added in the glass composition in order to stimulate the Ca/P component of hydroxyapatite (HA), the inorganic mineral of bone (Hench, 2013).

The oxide composition of 45S5 allows it to bond with both hard and soft tissues (Faure *et al.*, 2015). *In vitro* experiment using 45S5 glass, heterogeneous nucleation of the HA layers on the glass surface was observed indicating the process of mimicking bone mineralization during bone repair. This finding indicated the possibility to develop HA layer on the implant glass which later will provide interfacial bonding with living bone. During, *in vivo* experiment in mid shaft femur of rats, evidence of development of polycrystalline HA layer on the implant 45S5 glass was observed to form bonding between collagen fiber (Hench, 2016). 45S5 bioactive glass has been widely used since mid 1980s in clinical treatments in

powder form as regenerative bone filler with product names such as Perioglas® and Novabone® (Novabone Corporation, Alachua Florida) (Jones *et al.*, 2007). It has been used clinically in medical and dental application since then (Hench *et al.*, 2014).

45S5 bioactive glass powder was first synthesized *via* conventional melt derived route. Melt derived route is a simple method and able to produce BG in massive production (Ma *et al.*, 2010). Bioactive glass produced *via* melt derived is fully amorphous without existence of other crystalline phase. The amorphous phase is obtained due to the sudden cooling effect after quenched (Aguilar-Reyes *et al.*, 2017). The bioactive glass produced *via* melt derived also exists in more disordered three-dimensional glass structure. The fully amorphous glass structure induced higher ability in formation of apatite layer compared to crystalline phase due to lower solubility that might be due to strong bonding between atoms in crystal lattice (Dziadek *et al.*, 2016). This route requires mixing stoichiometric amounts of oxides which is high purity silica (SiO_2), calcium carbonate (CaCO_3), sodium carbonate (Na_2CO_3) and phosphorus pentoxide (P_2O_5). The mixture will then be melted at high temperature 1450 °C (Hench *et al.*, 1971). Available literature also indicate range of melting temperature to synthesize 45S5 bioactive glass from above oxides, melted at 1400 °C for four hours (Lefebvre *et al.*, 2008; Yang *et al.*, 2013) and 1380 °C for two hours (Zarifah *et al.*, 2015). The molten glass was then subsequently quenched in graphite (Yang *et al.*, 2013) or stainless steel (Aguilar-Reyes *et al.*, 2017) mold to obtain glass block or in water to obtain glass frit (Zarifah *et al.*, 2015). In order to obtain glass powder, the glass frit or even glass block was crushed and ground *via* mortar or milling process (Yang *et al.*, 2013; Araújo *et al.*, 2015). Synthesis of BG powder through melt derived is an alternative method to obtain glass without destructing the amorphous network structure. The mixture of reactants will be